

Appl. No.: 10/674,750
Filed: September 30, 2003
Page 2

Amendments to the Specification:

Please amend the paragraph beginning on page 16, line 2, to read as follows:

A 30 µl sample of freshly drawn full blood from a healthy human male was obtained and diluted in 1000 µl of purified water (~~Direct Q™~~ DIRECT Q™, ~~Millipore™~~ Millipore, 17 MOhm) in a 1.5-ml plastic vial, and centrifuged at 2200 g (6000 rpm) to obtain plasma. An additional aliquot of the blood was taken and prepared for optical dark-field microscopy. The plasma was transferred to a 4-ml glass vial with a plastic cap and a teflon liner. An aliquot of plasma was again retained for dark-field microscopy. The vial and contents were subjected to heating at a temperature of 120°C and 20 psi of pressure for two hours. An aliquot of the heat-treated plasma was prepared for dark-field microscopy.

Please amend the paragraph beginning on page 19, line 2, to read as follows:

The quantitative analysis of proteins in the proteon samples was carried out by two different protein assays obtained from ~~BIO-RAD™~~ Bio-Rad Laboratories and ~~SIGMA™~~ Sigma according to the manufacturers' protocols. Samples of blood as described in the Example 1 were exposed to different temperatures and pressures. Results of the experiments are shown in Table 4.

Please amend the paragraph beginning on page 20, line 19, to read as follows:

An aliquot of blood pre- and post-treatment (see Example 1) was purified using the ~~DNeasy Tissue Kit™~~ DNEASY TISSUE KIT™ (Qiagen) according to the manufacturer's standard protocols for animal blood and bacteria. After final elution, samples were loaded on 1% agarose gel. DNA bands were visualized with ethidium bromide. DNA was detected in pre-treatment sample and undetected in the post-treatment sample.

Please amend the paragraph beginning on page 20, line 24, to read as follows:

Similar results were obtained using a ~~High Pure PCR Template Preparation Kit™~~ HIGH PURE™ PCR template preparation kit from Roche for isolation of Nucleic Acids followed by fluorometric quantitation of double-stranded DNA using the ~~PicoGreen dsDNA Quantitation~~

RTA01/2160570v1

Appl. No.: 10/674,750
Filed: September 30, 2003
Page 3

~~Reagent™~~ PICOGREEN™ dsDNA quantitation reagent from Molecular Probes and a ~~TECAN Spectrafluor-Plus~~ TECAN SPECTRAFLUOR PLUS™ equipped with ~~DeltaSOFT~~ DELTASOFT™ software for detecting fluorescence (excitation at 485 nm and emission at 535 nm). See Table 4.

Please amend the paragraph beginning on page 22, line 1, to read as follows:

Optical observation of proteons was performed with an ~~Olympus™~~ Olympus microscope fitted with a 100-W mercury lamp illumination source, a polarizer, a Naessens dark-field condenser (COSE Corp., Canada) and a 100× objective (oil, NA 1.4). The dark-field images were directed to a ~~DEI-470T Optronics CCD Video Camera System™~~ DEI-470T Optronics™ CCD video camera system (Optronics Engineering, CA) utilizing the methods described in Vodyanoy *et al.* (1994) *Langmuir* 10:1354-1357. A direct count of proteons was used to determine their concentrations in liquid samples, and ~~Image Pro™~~ IMAGE PRO™ (Creative Software, Inc.) was used to quantify the number of proteons.

Please amend the paragraph beginning on page 22, line 29 , continuing through page 23, line 17, to read as follows:

Samples of proteons grown at the suppressive presence of 8 M Urea were subjected to dialysis using a ~~Pierce Slide-A-Lyser 10K™~~ SLIDE-A-LYSER 10K™ (20h, 5 L, 20°C) according to the manufacturer's instructions. The number of proteons found by dark-field microscope increased significantly. Samples of plasma treated with 120°C heat and 20 psi pressure and urea at concentrations of 0.01-8 M were taken. Polyacrylamide gel electrophoresis was carried out on each sample with a 4-20 % Tris-HCl ~~Ready-Gel™~~ READY GEL™, Bio-Rad according to the manufacturer's protocol). The control (proteons without chaotropic compounds) showed two characteristic bands of 14,400 and about 8,000 D. The experimental samples (proteons in the presence of a chaotropic compound) displayed a diffuse distribution of proteins or fragments of proteins with no sharp bands of proteins of the high molecular mass range. As the concentration of urea increased, the intensity of the diffusion staining decreased, and almost fully disappeared at the 8 M concentration of urea. The proteon sample displays a 14,400 D

RTA01/2160570v1

Appl. No.: 10/674,750
Filed: September 30, 2003
Page 4

band that coincides with a similar band found in plasma. When guanidine hydrochloride or urea was added to proteons produced without the chaotropic compounds, heat of 120°C and pressure of 20 psi resulted also in a great reduction of number of proteons visible by dark-field microscopy. Dialysis of these samples restored the population of the proteons. Gel electrophoresis of proteons and plasma treated with 120°C heat and 20 psi pressure at the presence of urea, and then dialyzed, reveals two bands of 14,400 and about 8,000 D in all samples, including those before and after dialysis.

Please amend the paragraph beginning on page 26, line 23, to read as follows:

The impact of PNCs upon viability of various cultured cells was investigated using the ~~tetrazolium~~ tetrazolium salt (MTT) cell proliferation assay. RG2 (mouse brain glioma), F98 (rat brain glioma), Hs683 (human brain glioma), CTX TNA2 (rat transfected astrocyte), H9c2[2-1] (rat heart myocardium), 27FR (rat skin fibroblast), and SVGP12 (human brain astroglia) cells were obtained from American Type Culture Collection (ATCC™) and maintained as recommended by ~~ATCC~~ ATCC™. MTT cell proliferation assays are commercially available. See, e.g., MTT Cell Proliferation Assay from ATCC™.

Please amend the paragraph beginning on page 26, line 30, continuing through page 27, line 5, to read as follows:

Cells were plated in D5648 (~~Sigma™~~ Sigma) + 10 % FCS (~~HyClone™~~ HyClone) in polystyrene 96-well plates at a density 3×10^3 cells per well. Twenty-four hours after plating, the medium was replaced with DMEM with either staurosporine (100 μ l, 1 μ M) or PNC (aliquots, 100 μ l, 5×10^9 - 3×10^{11} PNC/ml). PNCs were isolated from blood obtained from shark, dog, and rabbit using the ultrafiltration protocol described in Example 5. PNCs were autoclaved at 120°C and 20 psi for 15 minutes before adding to the cell cultures.

RTA01/2160570v1

Appl. No.: 10/674,750
 Filed: September 30, 2003
 Page 5

Please amend the paragraph beginning on page 27, line 6, to read as follows:

After 20 hours of treatment, a 20- μ l aliquot of ~~tetrazolium~~ tetrazolium salt (MTT, 5 mg/ml in PBS) was added to the wells, and interaction was allowed to proceed for 4 hours at 37°C. MTT was reduced in metabolically active cells to form purple formazan crystals, which were dissolved by DMSO and quantified by a plate reader (~~BioRad™~~ Bio-Rad). For each cell type, a linear relationship between cell number and absorbance is established, enabling accurate, straightforward quantification of changes in proliferation.

Please amend Table 1, page 8, to read as follows:

Table 1. Conformational diseases. See Carrel and Lomas (1997) *Lancet* 350:134-138; Carrell and Gooptu (1998) (1998) *Curr. Op. Struct. Biol.* 8:799-809.; Soto (2001) *FEBS Letters* 498:204-207; Jaikaran and Clark (2001); Ursini *et al.* (2002) *Trends Mol. Med.* 8:370-374; Kannan *et al.* (1988) *J. Biol. Chem.* 263:13766-13773; Schluter and Drenckhahn (1986) *PNAS* 83:6137-6141; Gerner *et al.* (2002); Davis *et al.* (2002) *Lancet* 359:2242-2247; Fernandez *et al.* (2001) *Atherosclerosis* 158, 103-111(atherosclerosis); Papalexis *et al.* (2001) *Mol. Biochem. Parasitology* 115:77-86; Esievo *et al.* (1984) *Veterinary Parasitology* 15:181-185; Igoc *et al.* (2002) *Clin. Microb. Newsletter* 24:69-70; Kreidl *et al.* (2002) *J. Am. Coll. Surgeons* 194:387; Beckers (2001) *Netherlands J. Med* 58:204-207 (autoimmune diseases); Ismeno *et al.* (1999) *Int'l J. Cardiology* 69:179-183; Klibansky *et al.* (1966) *Toxicon* 3:213-216; Seibert *et al.* (2003) *Toxicon* 41:831-839; Szabo *et al.* (2002) *Thrombosis Research* 107:357-363; Kaioumova *et al.* (2001) *Chemosphere* 43:801-805.

| Protein | Disease |
|---------------|--|
| Hemoglobin | Sickle cell anemia and aggregates Heinz bodies in aged erythrocytes Unstable hemoglobin inclusion body hemolysis Drug induced inclusion body hemolysis Aggregates regulate apoptosis in cancer patients Atherosclerosis Malaria Infections Auto-immune disorders Toxic reactions Internal bleedings |
| Prion protein | Creutzfeld-Jacob disease (CJD) New variant CJD |

RTA01/2160570v1

Appl. No.: 10/674,750
 Filed: September 30, 2003
 Page 6

| | |
|--------------------------|---|
| | Bovine spongiform encephalopathy (BSE) Gerstmann-Straussler-Scheinker disease Fatal familial insomnia Kuru |
| β -amyloid | Alzheimer's disease Down's syndrome Familial Alzheimer's |
| α -Synuclein | Parkinson's disease, Lewy bodies |
| Tau protein | Frontotemporal dementia, Pick bodies |
| Serpins | α_1 -antitrypsin deficiency -cirrhosis -emphysema Antithrombin deficiency -thrombosis C ₁ -inhibitor deficiency -angioedema |
| Neuroserpin | Neurodegenerative disease, Collins bodies |
| Glutamate repeats | Inherited neurodegenerative neurodegenerative disorders Huntington's Huntington's disease |
| Amylin | Diabetes type II |
| SOD | Amyotrophic lateral sclerosis |
| ApoB | Atherosclerosis |
| CFTR protein | Cystic fibrosis |
| Immunoglobulin | Systemic amyloid light chain amyloidosis |
| Amyloid light chain | Nodular amyloidosis |
| Serum amyloid A | Reactive systemic amyloid A amyloidosis Chronic inflammatory disease |
| Transthyretin | Senile systemic amyloidosis Familial amyloid neuropathy Familial cardiac amyloid |
| β_2 -microglobulin | Hemodialysis amyloidosis Prostatic amyloid |
| Apolipoprotein AI | Familial amyloid polyneuropathy Familial visceral amyloid |
| Cystatin C | Hereditary (Icelandic) cerebral angiopathy |
| Lysozyme | Familial visceral amyloidosis |

RTA01/2160570v1

Appl. No.: 10/674,750
Filed: September 30, 2003
Page 7

Please amend the paragraph beginning on page 32, line 22, to read as follows:

Experiments were carried out to compare the protecons obtained by the procedure described in the Example 1 with the nanobacteria isolated from blood by Kajander *et al.* (1996) *Mol. Biol. Cell* 7:3007-3007 using the ~~Nanocapture ELISA™~~ NANOCAPTURE™ ELISA, an Enzyme-Linked Immunosorbent Assay, for detection of nanobacterial antigens (Nanobac OY, Finland). The nanobacteria included in the ~~Nanocapture~~ NANOCAPTURE™ ELISA were used as a positive control. The Microplate Manager 4.01 Bio-Rad was used to obtain the results of the ELISA. The assay procedure was carried out following the manufacturer's recommendations. The reaction was considered to be positive when the absorbance was significantly higher ~~then~~ than the level of noise. Results of interaction of antibodies grown against nanobacteria with protecons and plasma are summarized in Table 11.

RTA01/2160570v1